AN AERIAL FIELD TRIAL EVALUATING SPLIT APPLICATIONS AND NEW FORMULATIONS OF *Bacillus thuringiensis* AGAINST THE SPRUCE BUDWORM (*Choristoneura fumiferana*) IN MAINE - 1986

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Authors
Henry Trial, Jr.
John B. Dimond

Maine Forest Service
DEPARTMENT OF CONSERVATION
Augusta, Maine 04333

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Henry Trial, Jr., Maine Forest Service, Old Town, Maine
and
John B. Dimond, Entomology Department, University of Maine, Orono, Maine

Abstract

Aerial applications of single and double (split) treatments of several standard and new formulations of *Bacillus thuringiensis* (B.t.) were evaluated against the spruce budworm infestation in eastern Maine in 1986. The most evident result was that double applications of 8 plus 8 BIU (Billion International Units) per acre were superior to single applications of 8 or 12 BIU. This was particularly evident on red spruce, a host species where good control of spruce budworm is often difficult to achieve. The highly concentrated formulations, Dipel 12L and Thuricide 64LV, applied as single applications of 12 BIU per acre did not provide uniformly favorable results. No difference was seen in efficacy between two variations of the formulation of Dipel 8L.

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Table of Contents

	Page
Introduction	1
Materials and Methods	1
Results	5
Discussion	11
Literature Cited	13
Appendix	15

LIST OF FIGURES AND TABLES

FIGURE		PAGE
1	1986 Maine B.t. Trial Block Locations	3

TABLE		
1	1986 Maine B.t. trials - treatments tested	2
2	Mortality of spruce budworm on balsam fir in experimental spray blocks, with analysis of variance	6
3	Mortality of spruce budworm on spruce in experimental spray blocks, with analysis of variance	7
4	Defoliation by spruce budworm on balsam fir in experimental spray blocks, with analysis of variance	8
5	Defoliation by spruce budworm on spruce in experimental spray blocks, with analysis of variance	9

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Introduction

The Maine Forest Service (MFS), University of Maine Entomology Department, Abbott Laboratories, Zoecon Corporation, and Champion International Corporation undertook a cooperative field test of the effectiveness of double (or split) applications and new formulations of Bacillus thuringiensis against the spruce budworm, Choristoneura fumiferana in 1986. The goals of the study were to reevaluate the effectiveness of a split application of B.t. against spruce budworm on mixed stands of red spruce and balsam fir and to compare the effectiveness of several newly developed B.t. formulations to a formulation used in recent operational spray projects. A split application B.t. trial was conducted in 1985, but poor spray deposits caused erratic results and a reevaluation of the split application strategy was recommended by the MFS. New formulations evaluated in this test were produced by Abbott Laboratories and Zoecon Corp. to address several aspects of B.t. development including potency, genetic strain, and product formulation technology.

Tests were conducted in several eastern Maine townships on land owned by Champion International Corp. during May, June, and July. The test was funded by the cooperators.

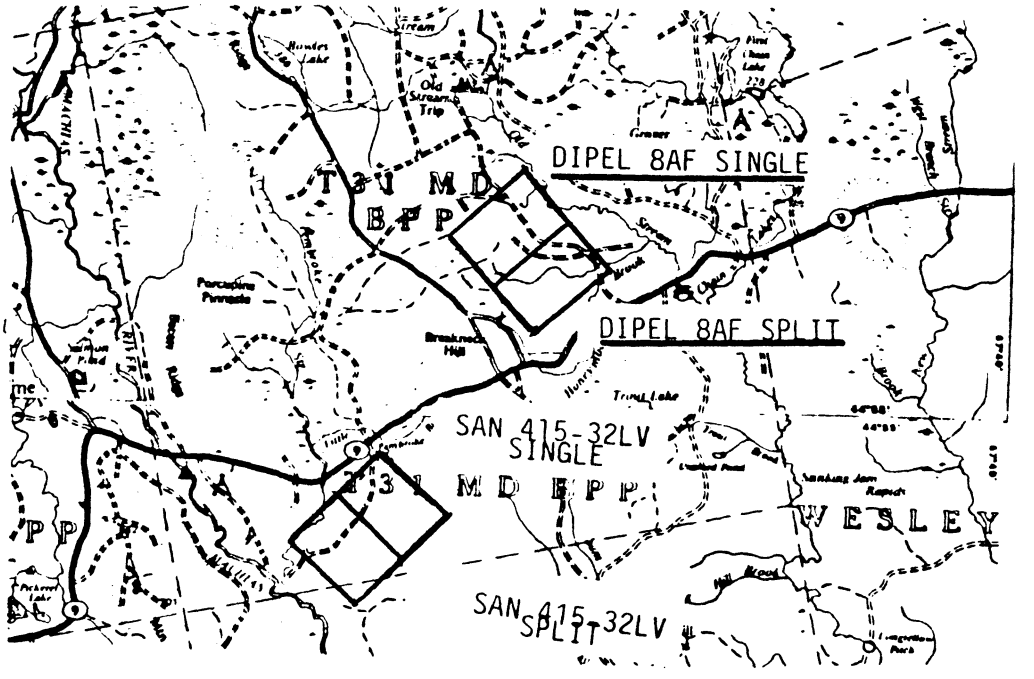
Materials and Methods

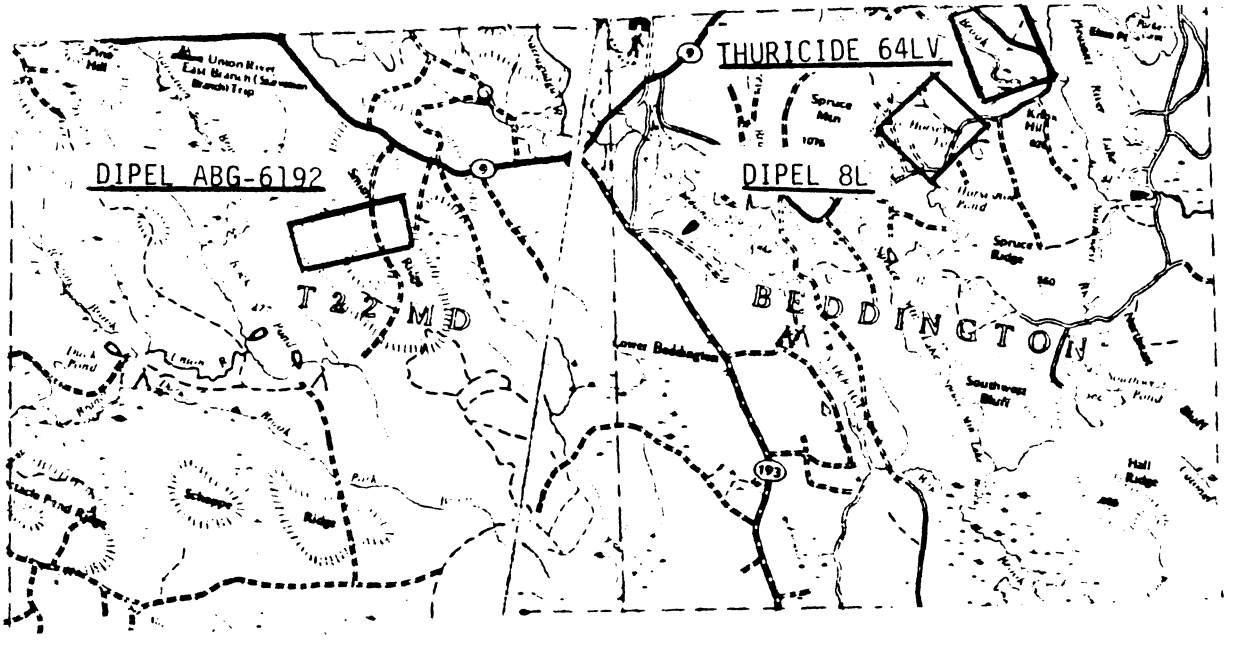
Six B.t. formulations, four provided by Abbott Laboratories and two by Zoecon Corp., were tested. All materials were applied as a single applications and two materials were applied as double (or split) applications for a total of eight spray regimes tested (Table 1). All formulations were sprayed undiluted. Dipel 8AF, San 415-32LV, and Thuricide 64LV have an aqueous base while Dipel 8L, Dipel ABG-6192 and Dipel 12L all have an oil base. Dipel 8L at a 12 BIU single application was used as an operational standard. Dipel ABG-6192, Dipel 12L, and Thuricide 64LV were new products to be compared to the standard. Dipel ABG-6192 does not contain emulsifiers and was thought to be less sensitive to water contamination than Dipel 8L. Dipel 12L and Thuricide 64LV represent attempts by Abbott and Zoecon respectively to produce effective, more concentrated formulations. Dipel 8AF and San 415-32LV were evaluated as 8 BIU single and as 8 BIU + 8 BIU split applications. Split applications of chemical insecticides have been shown to provide better protection than single applications especially on red spruce (Picea rubens Sarg.) and mixed spruce and fir (Abies balsamea (L.) Mill.). In this test, results in the split blocks were compared with the 12 BIU standard. The San 415-32LV formulation contains the NRD-12 strain of B.t. which has been shown to be more effective against budworm in the laboratory. Increased effectiveness of NRD-12 in a field situation could be shown if the single 8 BIU application of San 415-32LV compared favorably with the 12 BIU standards.

All single application blocks were 400 acres. Split application blocks were 600 acres, 300 acres of which were treated once and 300 acres treated

Table 1. - 1986 Maine B.t. trials - Treatments tested.

Insecticide	Manufacturer	Formulation Base	# Appl.	Rate BIU	Volume Oz./Acre
Dipel 8L	Abbott	Oil	1	12 BIU	24
Dipel 8AF	Abbott	Aqueous	1	8 BIU	16
Dipel 8AF	Abbott	Aqueous	2	8 BIU Twice	16 Twice
Dipel ABG-6192	Abbott	Oil	1	12 BIU	24
Dipel 12L	Abbott	Oil	1	12 BIU	16
Thuricide San 415-32LV	Zoecon	Aqueous	1	8 BIU	32
Thuricide San 415-32LV	Zoecon	Aqueous	2	8 BIU Twice	32 Twice
Thuricide 64LV	Zoecon	Aqueous	1	12 BIU	24





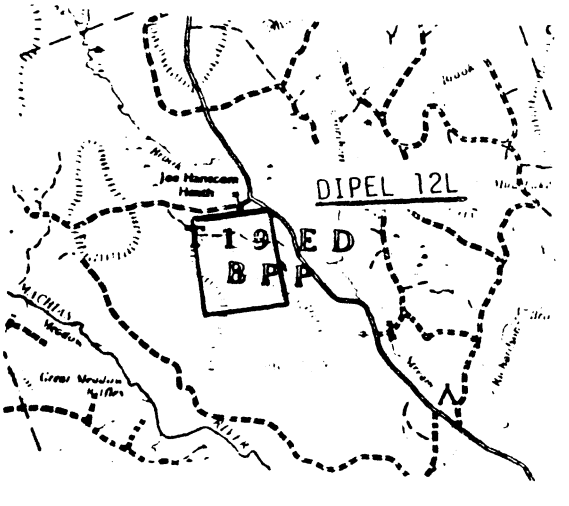


Figure 1.

1986 Maine B.t.
Trial Block Locations

twice. Blocks were located in several unorganized townships in central Hancock and Washington Counties (Figure 1).

All test blocks had been treated one or more times in operational projects in recent years. Blocks were predominantly softwood with a high percentage of red spruce and about 20 percent fir. Recent treatment had improved tree condition but stands generally remained in high hazard. Untreated check lines were located near treated blocks and contained similar conditions.

All treatments were made with a single Thrush Commander aircraft fitted with six AU 3000 Micronaire rotary atomizers with blades set at 35 . Spray block corners were marked with helium filled weather balloons or plastic jugs mounted on poles. A second aircraft, flying above the spray aircraft was used to assist in guidance and to provide monitoring of spray conditions.

Each spray block had 10 sample lines, 5 fir lines and 5 red spruce lines, established generally perpendicular to the spray swath direction. Sample lines consisted of 10 marked fir or red spruce. Trees were 25 to 100 feet apart and lines 500 to 800 feet long. The same sampling scheme was used for untreated check lines. Two blocks, Dipel 8L and Thuricide 64LV, contained insufficient fir to establish five lines. These two blocks each had only 24 fir sample trees. In statistical analysis, each 10-tree line was considered a replicate. This produced five replicates each for fir and spruce on most blocks. In the two blocks where fir was scarce, two replicates of 12 fir trees each were used. Spruce replicates remained at five.

An 18 inch branch was collected from the upper midcrown of each sample tree, two or three days prior to spraying to provide a prespray population estimate. Each branch was placed in a plastic bag with a branch identification card. All branches were transported to the MFS laboratory in Howland where larvae were counted by experienced laboratory workers.

The first spray application of the splits was timed to correspond to larval movement to red spruce buds. The second application was planned for the peak of the 5th larval instar. In single application blocks, spray was planned to occur when 70% of larvae reached the 4th larval instar. Samples of fir and spruce branches were collected from within or near the blocks every two days to assess development conditions. Larval and shoot development as well as larval position relative to spruce buds were assessed for each sample.

The first application of the splits was applied on both blocks on May 28th. Spraying was delayed until late afternoon by fog and low overcast. Blocks were sprayed in light winds (3-5 MPH) and temperatures near 60 F. Single application blocks were sprayed on May 29th beginning at 10:20 a.m. Spray had been delayed by morning fog. Winds were light (3-5 MPH) with cloudy skies at a temperature of 58 to 60 F. Scattered light showers occurred in the area of the single application blocks shortly after spraying ended for the day. The second application of the splits was made on June 9th. Spraying began at 5:15 a.m. with clear skies, temperature in the mid 50's, and moderate winds (8-12 MPH).

Spray deposit was assessed by microscopic examination of dyed spray droplets on fir foliage. Only water based formulations were assessed because a B.t.-compatible, oil-soluble dye was unavailable. Water based formulations were dyed with 0.02 percent by weight of erio acid red dye. Field sampling was done as soon after spraying as possible, but in some cases sampling was delayed until the following morning. Eight twigs were collected from the midcrown of 30 of the 50 fir sample trees in each block. These twigs were stuck in styrofoam holders which kept units separated and the holder then placed in cardboard boxes and taken to Orono to be frozen until foliage could be examined. When samples were taken from the freezer for examination they were thawed in the presence of anhydrous sodium sulfate to prevent moisture formation. When thawed, 10 needles were taken at random from each twig. Needles were placed between glass slides and examined for droplets on both upper and lower surfaces.

Two postspray population samples were collected at seven days following spraying and at budworm pupation. Branch samples were handled the same as prespray samples.

After surviving budworm had pupated and all feeding had ceased, defoliation was estimated. One midcrown branch was collected from each sample tree; starting from the top of the branch and working down one side and then the other, 50 shoots were rated using the Fettes method (Fettes, 1950). A defoliation percentage was calculated for each branch using the Fettes score.

Data were analyzed by analysis of variance. When analyzing population reduction and foliage saved by treatment, where percentages were used, the arcsine transformation was applied. Treatments were compared to checks and other treatments by orthogonal contrasts.

Results

The difference in prespray spruce budworm population density between treatments presented some problems in the analysis of data (Table 2). Because the three western blocks (Dipel 8L, ABG - 6192, Thuricide 64LV) had distinctly lower populations than the other treatments and the controls, data are expressed and analyzed as percent reduction between the prespray count and the final postspray count, with an arcsine transformation used with the statistical analysis.

Population reduction on fir

On fir (Table 2), all treatments produced significantly greater reductions in populations, compared to controls, except Dipel 12L and Thuricide 64LV. The two, single 8 BIU treatments (Dipel 8AF and Thuricide SAN415) were also not significantly different from controls at $p<0.05$, but this was barely the case; they were significant at $p<0.10$. It is clear from inspection of the upper part of the table that this statistical result was due to the fact that Dipel 8AF, as a single 8 BIU treatment, produced a favorable result but SAN415 did not. Both formulations were significantly better as double applications.

Dipel 8L as a single application of 12 BIU, on fir, appeared equal in efficacy to the double applications of 8 BIU. In comparison with Dipel 8L,

Table 2. Mortality of spruce budworm on balsam fir in experimental spray blocks, with analysis of variance. (Data are treatment means)

Treatments	Insects per branch Prespray	Postspray	Percent Reduction
Dipel 8AF single 8 BIU/a	20.7	3.5	83.1
Dipel 8AF double 8+8 BIU/a	19.2	2.4	87.5
Thuricide SAN415 single 8 BIU/a	16.2	5.2	67.9
Thuricide SAN415 double 8+8 BIU/a	24.3	1.4	94.2
Dipel 8L single 12 BIU/a	8.6	0.4	95.3
ABG - 6192 12 BIU/a	5.5	1.2	78.2
Dipel 12L single 12 BIU/a	20.7	8.0	61.4
Thuricide 64LV single 12 BIU/a	5.1	1.0	80.4
Check 1	19.9	4.9	75.4
Check 2	19.9	5.5	72.4
Check 3	28.1	10.7	61.9
Check 4	21.3	8.1	62.6

Contrasts	df	ANOVA Sum of squares	Mean square	F	
Single 8 BIU vs checks	1	205	205	4.02	non sig.
Double 8 BIU vs checks	1	2294	2294	44.98	sig.
Single 8 vs double 8 BIU	1	845	845	16.57	sig.
Dipel 8L vs checks	1	1052	1052	20.63	sig.
8L vs ABG - 6192	1	447	447	8.76	sig.
8L vs double 8 BIU	1	50	0	0.00	non sig.
Dipel 12L vs checks	1	72	72	1.41	non sig.
Thur. 64LV vs checks	1	78	78	1.53	non sig.
Residual	42	2137	51		

Table 3. Mortality of spruce budworm on spruce in experimental spray blocks, with analysis of variance. (Data are treatment means)

Treatments	Insects per branch Prespray	Postspray	Percent Reduction
Dipel 8AF single 8 BIU/a	15.4	3.2	79.2
Dipel 8AF double 8+8 BIU/a	18.2	2.4	86.7
Thuricide SAN415 single 8 BIU/a	13.3	5.6	58.0
Thuricide SAN415 double 8+8 BIU/a	17.1	1.7	89.9
Dipel 8L single 12 BIU/a	5.9	2.2	62.3
ABG - 6192 12 BIU/a	5.5	2.1	57.0
Dipel 12L single 12 BIU/a	9.8	7.8	19.7
Thuricide 64LV single 12 BIU/a	8.7	2.5	71.1
Check 1	9.8	6.0	38.3
Check 2	11.1	4.2	62.0
Check 3	16.9	6.5	61.7
Check 4	16.9	6.1	64.2

ANOVA

Contrasts	df	Sum of squares	Mean square	F	
Single 8 BIU vs checks	1	523	523	5.68	sig.
Double 8 BIU vs checks	1	3953	3953	42.96	sig.
Single 8 vs double 8 BIU	1	1202	1202	13.06	sig.
Dipel 8L vs checks	1	24	24	0.26	non sig.
8L vs ABG - 6192	1	0	0	0.00	non sig.
8L vs double 8 BIU	1	1599	1599	15.67	sig.
Dipel 12L vs checks	1	1998	1998	21.72	sig.*
Thur. 64LV vs checks	1	640	640	6.96	sig.
Residual	48	4400	92		

* This is significantly poorer than controls rather than better.

Table 4. Defoliation by spruce budworm on balsam fir in experimental spray blocks, with analysis of variance. Data are treatment means

Treatments	Expected % defoliation	Observed % defoliation	Exp - Obs/Exp * 100 (% foliage saved)
Dipel 8AF single 8 BIU/a	77	44	43
Dipel 8AF double 8+8 BIU/a	76	21	72
Thuricide SAN415 single 8 BIU/a	78	60	23
Thuricide San415 double 8+8 BIU/a	78	26	67
Dipel 8L single 12 BIU/a	31	13	58
ABG - 6192 12 BIU/a	31	10	68
Dipel 12L single 12 BIU/a	78	40	49
Thuricide 64LV 12 BIU/a	31	19	39
Check 1	77	76	1
Check 2	79	77	2
Check 3	81	80	1
Check 4	79	82	0

ANOVA

Contrasts	df	Sum of squares	Mean square	F	
Single 8 BIU vs checks	1	6325	6325	33.56	sig.
Double 8 BIU vs checks	1	29393	29393	155.97	sig.
Single 8 vs double 8 BIU	1	6337	6337	33.62	sig.
Dipel 8L vs checks	1	5540	5540	29.39	sig.
8L vs ABG - 6192	1	106	106	0.56	non sig.
8L vs double 8 BIU	1	209	209	1.11	non sig.
Dipel 12L vs checks	1	8391	8391	44.52	sig.
Thur. 64LV vs checks	1	2383	2383	12.64	sig.
Residual	42	7915	188		

Table 5. Defoliation by spruce budworm on spruce in experimental spray blocks, with analysis of variance.(Data are treatment means)

Treatments	Expected % defoliation	Observed % defoliation	Exp - Obs/Exp * 100 (% foliage saved)
Dipel 8AF single 8 BIU/a	45	20	56
Dipel 8AF double 8+8 BIU/a	39	16	59
Thuricide SAN415 single 8 BIU/a	39	38	3
Thuricide SAN415 double 8+8 BIU/a	39	11	72
Dipel 8L single 12 BIU/a	27	15	44
ABG - 6192 12 BIU/a	23	10	56
Dipel 12L single 12 BIU/a	35	30	14
Thuricide 64LV 12 Biu/a	45	27	40
Check 1	27	25	8
Check 2	32	36	0
Check 3	53	48	9
Check 4	58	61	0

ANOVA

Contrasts	df	Sum of squares	Mean square	F	
Single 8 BIU vs checks	1	2884	2884	8.57	sig.
Double 8 BIU vs checks	1	18096	18096	53.82	sig.
Single 8 vs double 8 BIU	1	4899	4899	14.57	sig.
Dipel 8L vs checks	1	1296	1296	3.85	non sig.
8L vs ABG - 6192	1	941	941	2.79	non sig.
8L vs double 8 BIU	1	3875	3875	11.53	sig.
Dipel 12L vs checks	1	581	581	1.73	non sig.
Thur. 64LV vs checks	1	876	876	2.61	non sig.
Residual	48	16140	336		

the ABG - 6192 was significantly less effective. While the direct contrast was not made, ABG - 6192 did not appear different from controls. In summary, on fir, the two double applications and the single application of Dipel 8L at 12 BIU/acre appeared successful. Other treatments did not or were of questionable efficacy.

Population reduction on spruce

The results of population reductions on spruce (Table 3) confirmed results on fir in some cases but were contradictory in others. Clearly, the two double applications of 8 BIU/acre gave the best results. The results of the single applications at 8 BIU/acre were also similar to fir, with Dipel 8AF showing efficacy but not SAN415. This success with Dipel 8AF at 8 BIU is difficult to reconcile with the failure of Dipel 8L and ABG - 6192 at 12 BIU. Thuricide 64LV provided a significant level of population control on spruce where it had not on fir, but in both cases the apparent levels of population reduction were less than in the better treatments.

Defoliation on fir

The measurements of defoliation and foliage saved in the sprayed blocks provide another measure of efficacy which is related to population reduction but which is measured independently. On fir, results (Table 4) suggest that all treatments saved significant amounts of foliage, compared to controls. This result is not unusual in B.t.-treated blocks where marginal effectiveness is often reflected in inapparent reductions in populations, but where sublethal effects of treatment save significant amounts of foliage. Following the rule-of-thumb that a criterion for success is the saving of at least 50 percent of the threatened foliage, then the two double applications of 8 BIU/acre and the single applications at 12 BIU of Dipel 8L and ABG - 6192 were successful. These results of damage to trees (Table 4) follow those of population reduction (Table 2) quite closely in relative efficacy on fir except for ABG - 6192. That treatment ranked second best in saving foliage but only sixth best in reducing populations.

Defoliation on spruce

Defoliation on spruce (Table 5) followed patterns similar to those on fir (Table 4). However, variability in the spruce data resulted in statistical non-significance in spruce where differences of the same magnitude were significant in fir. It can be concluded that the results are strengthened because of the agreement between fir and spruce. The same treatments tended to be more successful or less successful on both hosts.

Spray droplet deposit and weather

Aerial spray tests of the type carried out here are usually not well controlled experiments, and factors other than the actual treatments can influence results. The major factors that cannot be controlled are related to weather. Different wind and temperature regimes can result in different proportions of the spray clouds settling on the target foliage. Differences in rainfall after treatment can affect persistence of spray deposit. Monitoring of the first of these variables in these tests did not produce much useful information. In the first place, only the water-based formulations

could be dyed and monitored. In the second place, the counts of spray droplets on needles did not appear to relate well to efficacy.

Rainfall may have influenced some results. The second day of spray application ended in late morning with isolated, brief showers, and one of these was seen to enter the spray block treated with Dipel 12L as the last spray swaths were being flown. An examination of blocks sprayed that morning showed that the Dipel 12L block was the only one with wet foliage. Rain was apparently not a factor in the other treatments.

The droplet counts on needles are presented graphically in Appendix I. Note that the vertical axes of the graphs differ in scale so that they are not directly comparable in visual inspection. The values for mean drops per needle are comparable, however. It can be seen (Appendix I) that, for SAN415, droplets per needle in the first application of 8 BIU were 0.600 and 0.517 for each half of the block; the comparable values for Dipel 8AF were much lower, 0.133 and 0.242. Yet, Dipel 8AF gave superior results as a single 8 BIU treatment. In the second applications of the double treatments of these formulations, Dipel 8AF showed greater spray deposit, 0.217 droplets per needle, than SAN415 at 0.142 droplets. Yet, the second application of SAN415 appeared to provide a greater increase in protection than the second application of 8AF. The erratic spray deposit measured in the block treated with Thuricide 64LV (Appendix I) was also examined and the efficacy data was divided between one third of the trees with greatest deposit and two thirds of the trees with less deposit. Comparing these two groups, no relation of efficacy to measured spray deposit was found; the heavier-deposit group of trees had less percent population reduction and greater defoliation than the lighter-deposit group of trees. From these counts of spray droplets little can be concluded other than that all the spray blocks involved and most of the sample trees received spray deposit.

Discussion

The results of these tests are clear in some cases and less clear in others. One contributing factor to this was the declining trend in budworm populations in the region. Declining populations tend to be spotty and irregular making it difficult to predict infestation levels. Some treatment blocks had lower levels of infestation than had been predicted and some did not. Uneven population levels between treatments make direct comparisons of treatment effects difficult and reduce our confidence in comparing low population blocks to others. Unfortunately, all four control blocks had high populations which did not allow for good comparisons with the treatments applied to low-population blocks. A second factor was scarcity of balsam fir host trees in two treatment blocks resulting in only two replicates for fir in those blocks. With few degrees of freedom, this allows little confidence in the statistical analyses of the results on fir.

Double applications

The clearest result is the improved efficacy of double applications of 8 BIU/acre over single applications of 8 BIU on both fir and spruce. Whether a double 8 BIU application was superior to a single 12 BIU application was less

clear, however. On fir, either Dipel 8L or ABG - 6192 at single applications of 12 BIU looked about equal to the double 8 BIU treatments, when looking at population reduction or at defoliation. But on spruce, the double applications looked superior to all single applications. The differences involved here are not great and may be of little biological importance, however, they do fit a pattern that could be anticipated from past experience. Morris (1984) compared efficacy of single and double applications of both Dipel and Thuricide products in Ontario and reported no advantage in the double application. His observations were restricted to fir, however. Many reports, on the other hand, have noted improved efficacy of double applications of chemical insecticides but particularly on red or black spruce and less so on fir. The present results appear to follow this pattern. A single, well-timed application of B.t. or chemical insecticide may be as efficacious as a double application on fir. On red or black spruce, where timing of treatment is much more difficult, double applications are superior. Double applications of B.t. should be considered in future spray operations where red or black spruce are principal targets.

Dipel 12 L and Thuricide 64 LV

Another comparison of interest within the experiment was Dipel 8L and ABG-6192, which are small variations on the same formulation. Four different comparisons were considered: mortality on fir, mortality on spruce, defoliation on fir, defoliation on spruce (Tables 2-5). Of these four comparisons, there was a significant difference in only one, mortality on fir. The low replication on fir in the Dipel 8L block could lead to high experimental error in the fir comparisons. Because of this, and because 3 of the 4 comparisons between 8L and ABG-6192 showed no difference, there appears to be no convincing difference in efficacy. The results remain less than completely clear, however.

Dipel 12L and Thuricide 64LV showed significant efficacy above levels in controls in only 1 or 2 of the 4 comparisons described in the paragraph above. For this reason, their performances cannot be evaluated as successful. Rainfall immediately after application may have been a factor in the unsatisfactory performance of 12L, but that was probably not a factor with any other treatments. These two products appear to require additional development or testing before they can be recommended for control of spruce budworm.

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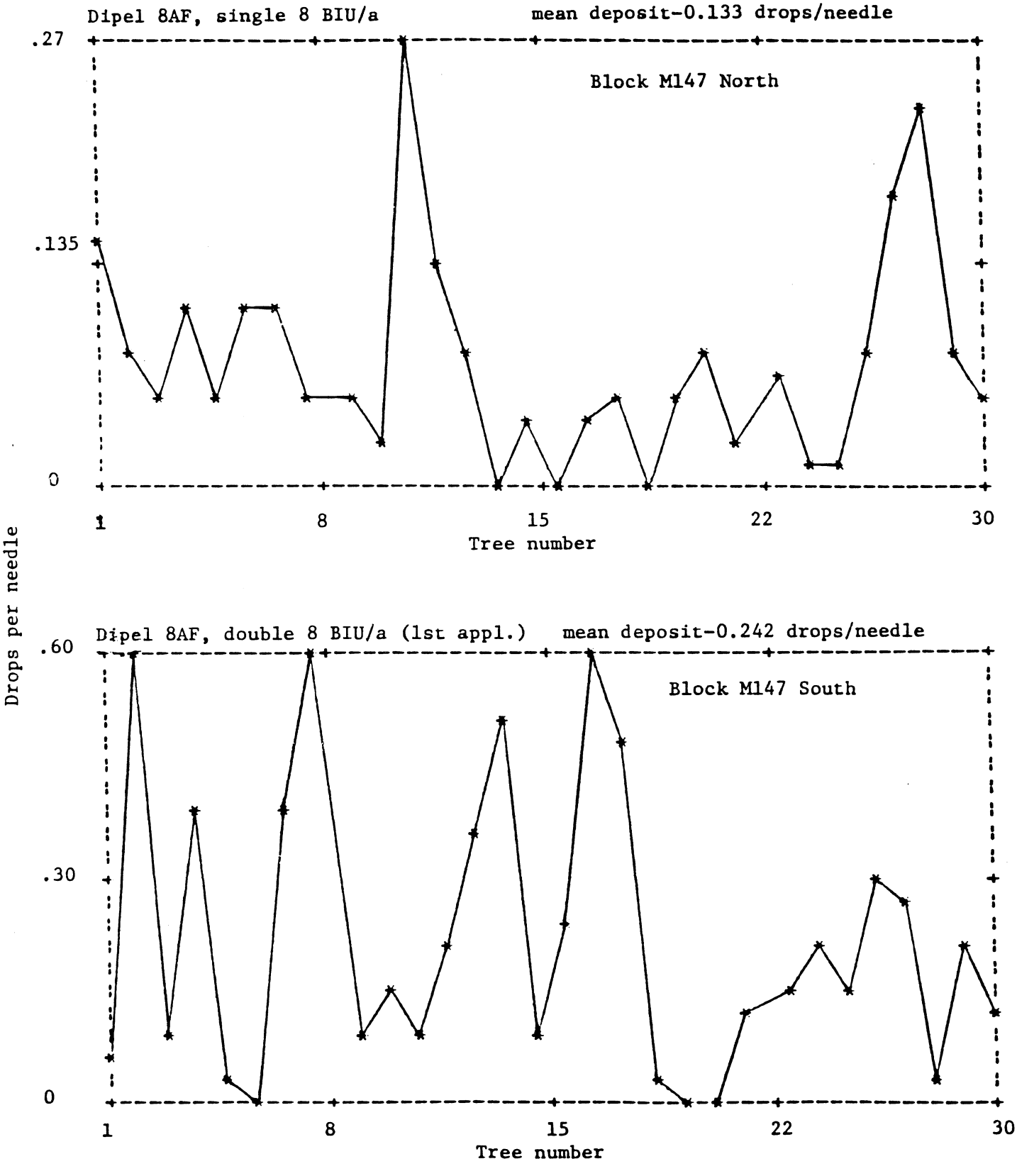
A P P E N D I X

APPENDIX I. Spray droplets per needle counted on 30 trees each in spray blocks treated with water-based sprays.



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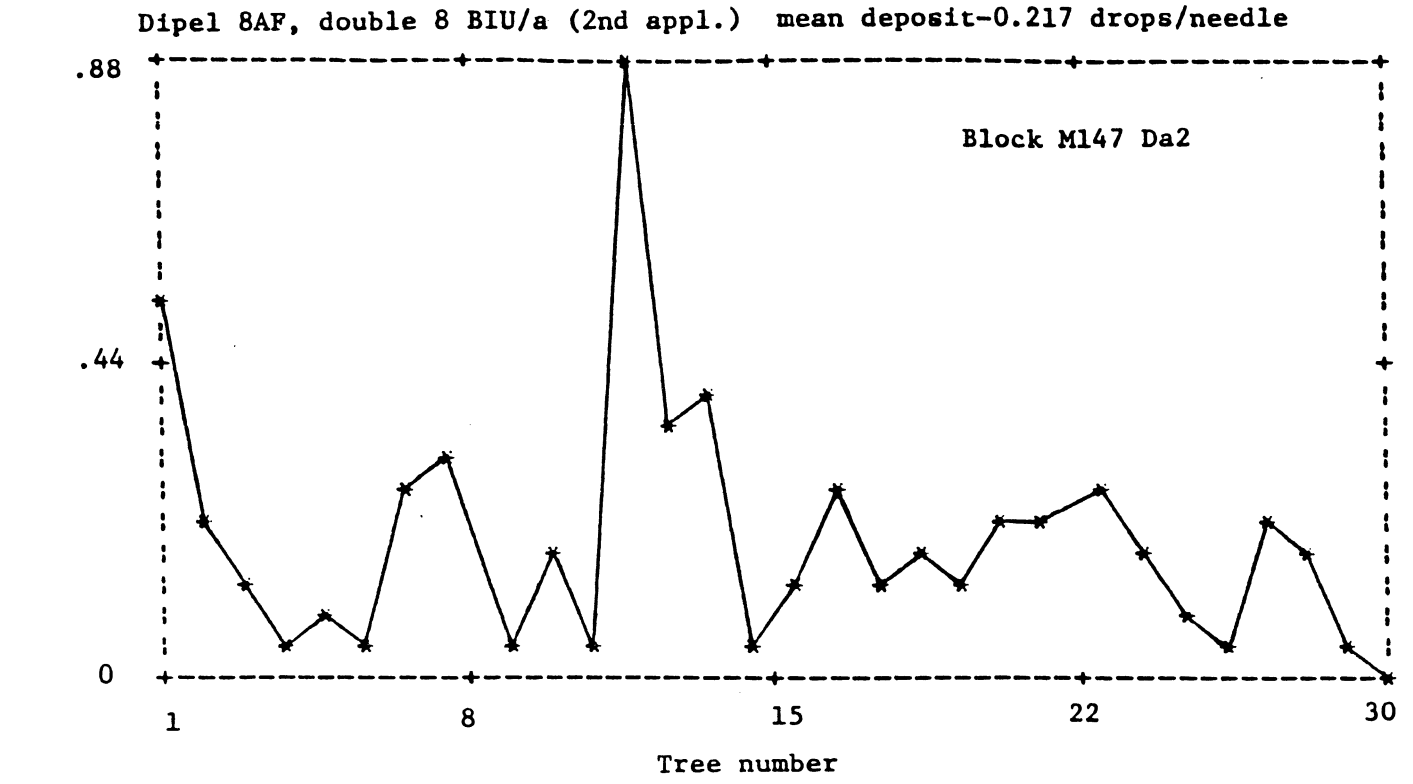

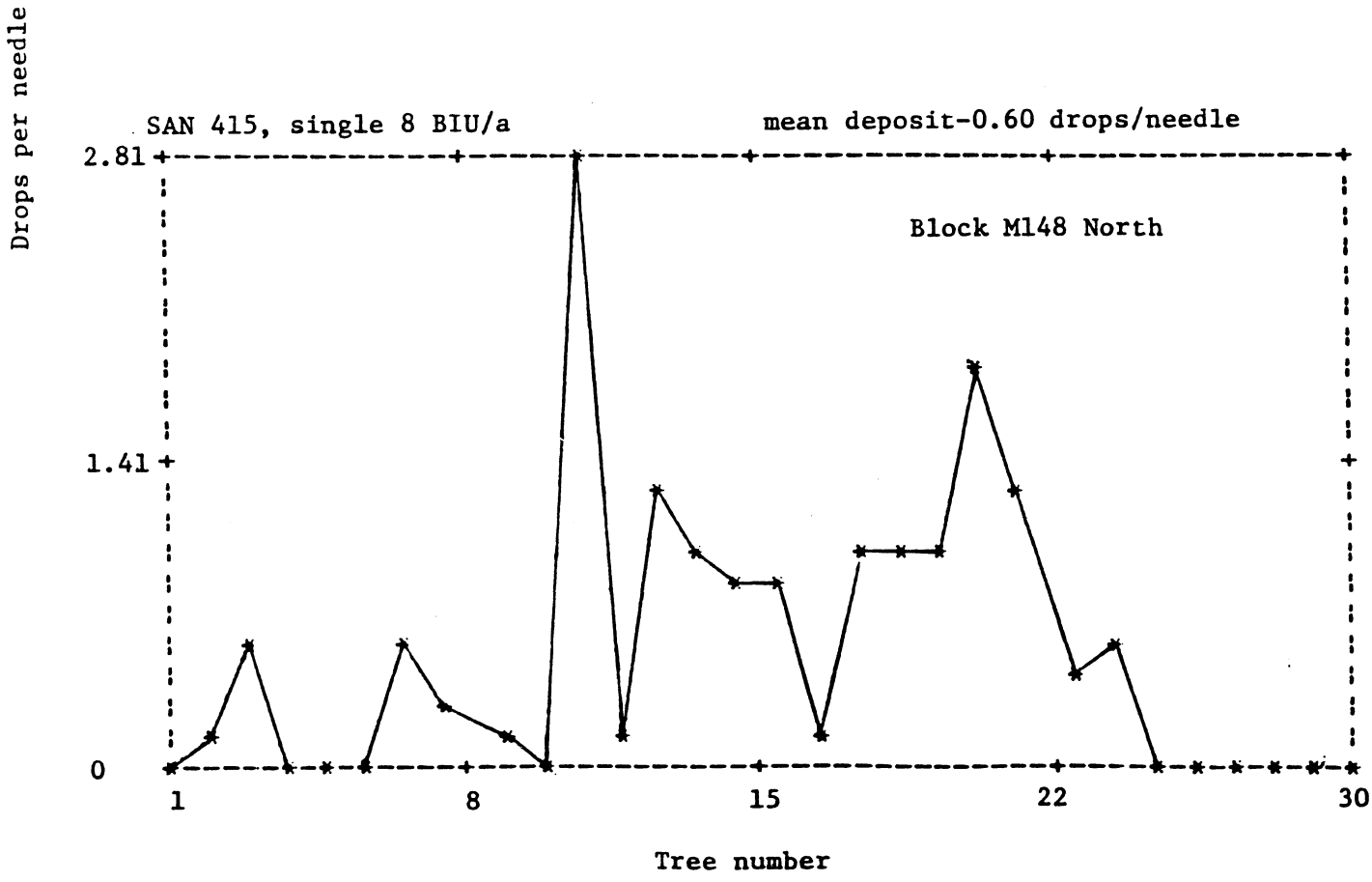

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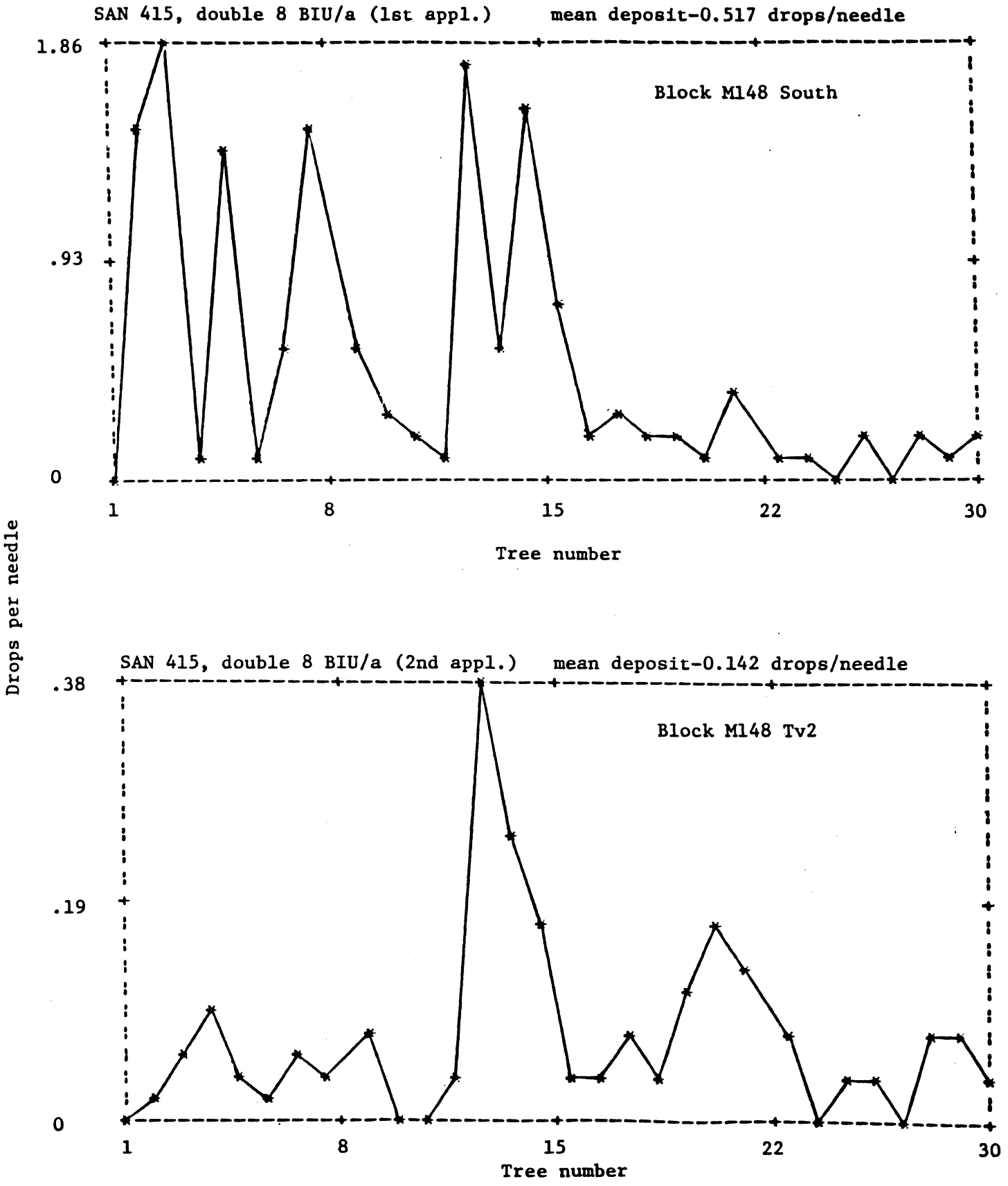

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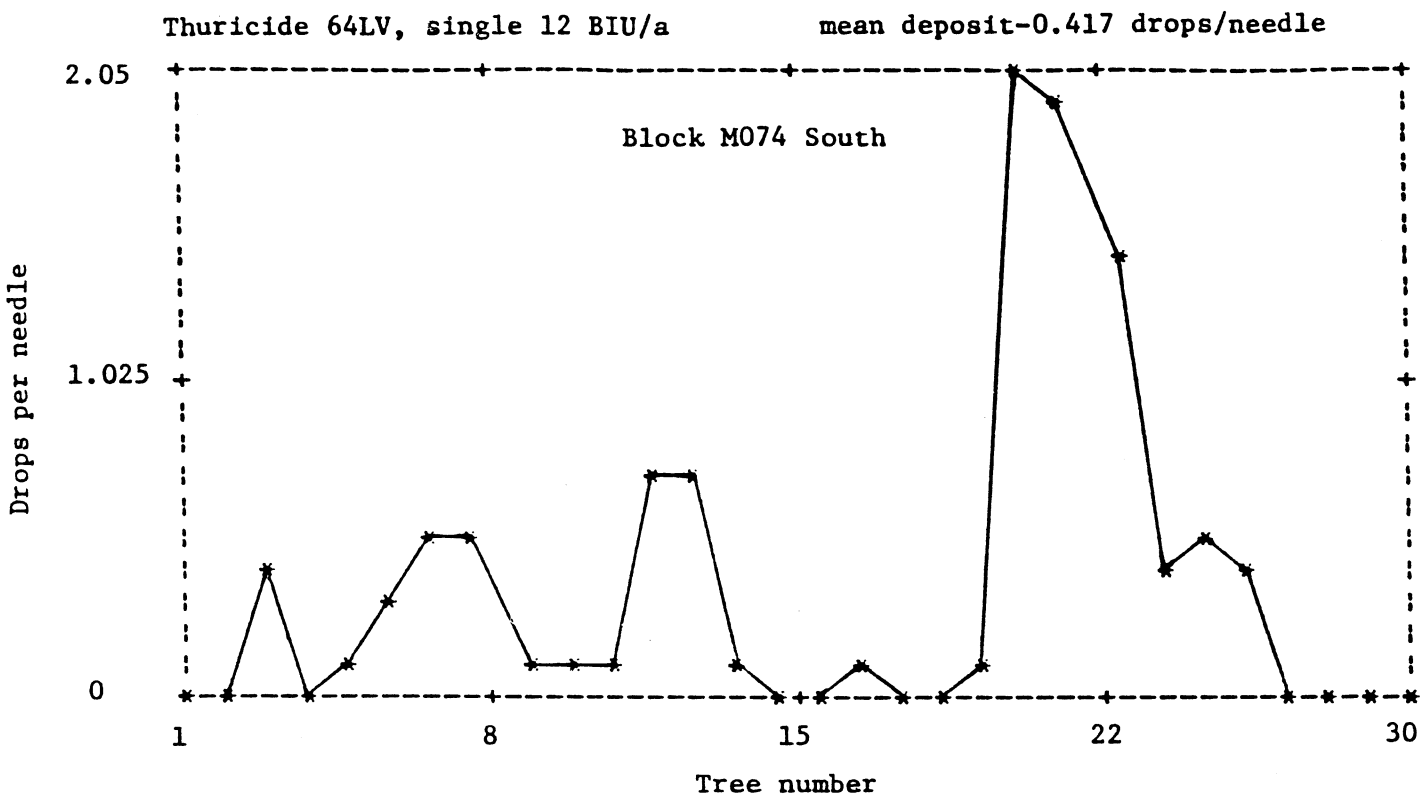

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